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Short communication

Propranolol inhibits the human ether-a-go-go-related gene potassium channels

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Abstract

Propranolol is a noncardioselective beta-adrenergic antagonist that has been recently reported to prolong the QTc interval on the surface electrocardiogram in humans when overdosed [Farhangi, V., Sansone, R.A. (2003). QTc prolongation due to propranolol overdose. Int. J. Psychiatry Med. 33, 201–202.]. To examine the underlying mechanisms for these clinical findings, we studied the effects of propranolol on the human cardiac potassium channels encoded by the *ether-a-go-go*-related gene (ERG) using the whole cell voltage-clamp technique. We found that propranolol blocked hERG currents in a concentration-dependent manner with an IC_{50} of 9.9±1.3 μM which is relevant to the predicted plasma level of propranolol in this case report. The present study demonstrated that propranolol can inhibit hERG channels. The interaction between propranolol and hERG channels could lead to delayed cardiac repolarization and might be a molecular mechanism for the previously reported QTc prolongation when propranolol is overdosed.

Keywords: HERG (human ether-a-go-go related gene); QTc interval prolongation; Propranolol

1. Introduction

Drug-induced prolongation of the QTc interval can lead to the development of a severe ventricular arrhythmia, Torsade de Pointes (TdP) which may cause sudden death. Therefore, acquired QT prolongation has become a major safety consideration for the clinical development and subsequent use of drugs. The QT interval is a reflection of the underlying action potential duration of ventricular myocytes. One of the most important currents that are critically involved in cardiac action potential repolarization is I_{Kr}. The human *ether-a-go-go*-related gene (hERG) encodes the major protein underlying I_{Kr} (Sanguinetti et al., 1995), and mutations in hERG account for the chromosome 7-linked form of inherited long QT syndrome (Viskin et al., 1999; Ficker et al., 2000). The hERG channel is inhibited by a variety of compounds known to prolong the QTc interval. Many important new therapies, such as terfenadine and cisapride have been withdrawn from the market because of a number of deaths caused by this unwanted side effect. Propranolol as a noncardioselective beta-adrenergic blocker has been used for a long period of time in the clinic. Just recently, propranolol was reported to prolong QTc in a patient when accidentally overdosed (Farhangi and Sansone, 2003). To investigate the mechanism behind these clinical findings, we examined the effects of propranolol on the cloned hERG potassium channels heterologously expressed in Chinese hamster ovary (CHO-K1) cells.

2. Materials and methods

2.1. Cell preparation

The hERG potassium channels were stably expressed in the CHO-K1 cells. The CHO-K1 cells were maintained in cell media

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that contained 90% Iscove's Modified Dulbecco's Medium, 10% fetal bovine serum, 1% HT Supplement (10 mM Sodium Hypoxanthine and 1.6 mM Thymidine), 1% NEAA (Non-Essential Amino Acids Solution), penicillin G sodium 100 units/ml, and streptomycin sulfate 100 μg/ml and geneticin 500 μg/ml. Confluent cells in flasks were rinsed once with PBS (Phosphate-Buffered Saline) prior to passage. The flasks were incubated with VERSENE (EDTA) 1:5000 for 5 min at 37 °C to detach the cells from the flasks. Cells used in electrophysiology experiments were plated on glass coverslips 24–48 h prior to use.

2.2. Voltage-clamp recording of hERG currents

hERG Currents were recorded using whole-cell voltage-clamp technique (Hamill et al., 1981) with an MultiClamp 700 A amplifier (Axon Instruments). Voltage clamp protocols were controlled via PC using pClamp9 (Axon Instruments) acquisition and analysis software. Borosilicate glass patch pipettes were pulled to obtain a tip resistance of 2 to 4 MΩ when filled with (mM): KCl 126, $MgSO_4$ 2, $CaCl_2$ 0.5, EGTA 5, Mg-ATP 4, and HEPES 25 (pH7.2). External bath solution consisted of (mM) NaCl 150, $CaCl_2$ 1.8, KCl 4, $MgCl_2$ 1, glucose 5, and HEPES 10 (pH7.4). The temperature was controlled at 25±0.5 °C for all experiments using a temperature controller (SHM-6 Multi-Line Solution Heater) from Warner Instruments. The currents were stable for up to 45 min.

Propranolol (Sigma Chemical, St. Louis, MO) was prepared as a 100 mM stock solution in DMSO (Dimethyl Sulphoxide) and stored at −20 °C. On the day of experiments, the stock solution was diluted to the desired concentrations with bath solution.

3. Results

The effects of propranolol on hERG channels are illustrated in Fig. 1. In these experiments, the cells were held at −80 mV, then stepped to +20 mV for 400 ms followed by a second pulse to −40 mV for 400 ms to produce large, slowly deactivating tail currents characteristic of hERG (Sanguinetti et al., 1995). This pulse protocol was continually repeated every 10 s during the entire experiment. hERG currents were recorded in control condition and during the application of propranolol. As shown in Fig. 1A and B, hERG tail currents were blocked by propranolol in a concentration-dependent manner with an IC_{50} value of 9.9±1.3 μM (n=4–8 cells at each concentration). Fig. 1C shows the time course of hERG tail current inhibition by propranolol at 10 μM. After addition of 10

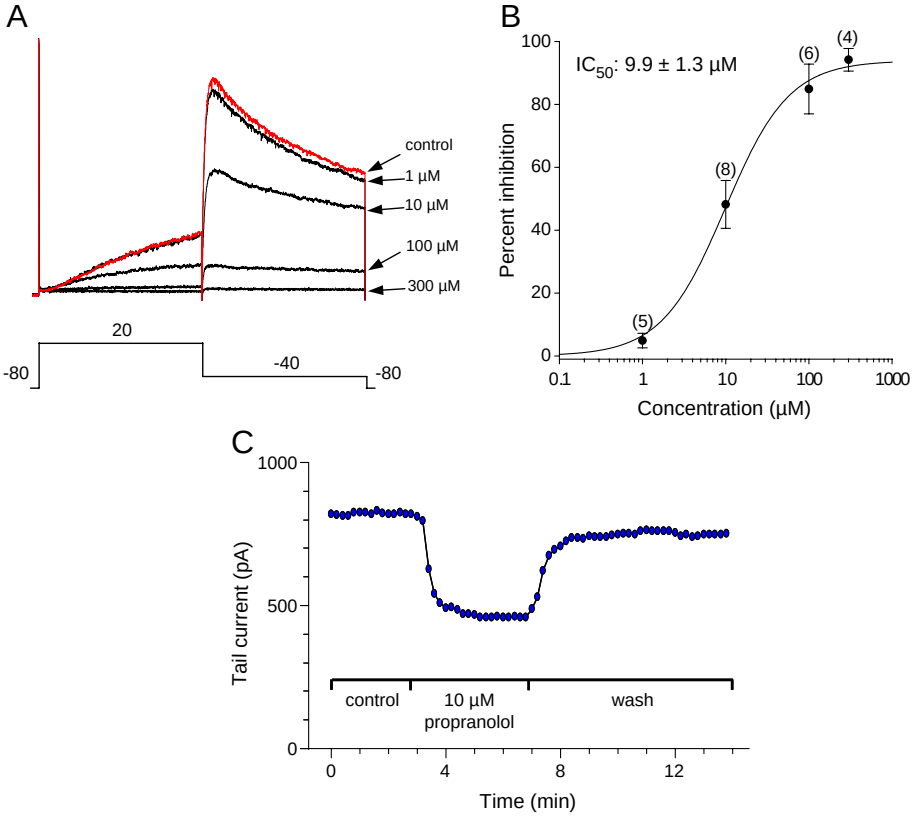

Fig. 1. Inhibition of hERG channels expressed in CHO-K1 cells by propranolol. A, representative current traces recorded from the same cell under control condition and after perfusion with propranolol (1, 10, 100 and 300 μM) are displayed. The cell was held at −80 mV, and then stepped to +20 mV for 400 ms followed by a second pulse to −40 mV for 400 ms before returning to the holding potential of −80 mV. This pulse protocol was repeated at 10-s intervals as propranolol was perfused. B, concentration-dependent relationship for the effect of propranolol on hERG peak tail currents. Error bars denote S.D. (n=6–9 cells). The IC_{50} yielded 9.9±1.3 μM. C, time curse of hERG peak tail current inhibition by 10 μM propranolol. After a control period of 10 min, currents decreased rapidly upon perfusion with the drug solution and reached the steady-state inhibition within 3 min. This inhibitory effect was reversible after washing the cells with control solution.

μM propranolol, hERG channel currents were blocked rapidly, and steady-state inhibition was reached within 1 min. The inhibitory effect of propranolol on hERG was reversed when the cell was washed with control bath solution.

Propranolol had no effect on tail current kinetics as is shown in Fig. 2. The cells were clamped at a holding potential of −80 mV. Depolarizing pulses were applied for 400 ms to voltages between −80 mV and +50 mV in 10 mV increments, and tail currents were recorded during a constant repolarizing step to −40 mV for 400 ms. Families of current traces from one cell are shown for control condition and after exposure to 10 μM propranolol shown in Fig. 2A and B, respectively. The currents activated at potentials greater than −50 mV and reached a peak at 0 mV and then decreased at more positive potentials due to inactivation (Sanguinetti et al., 1995), giving the I–V relationship its typical bell-shaped appearance (data not shown). The peak tail currents, measured during the second repolarizing step of voltage protocol, increased with voltage steps from −40 to +20 mV and then plateaued for test pulse potentials positive to +20 mV (Fig. 2C). In this experiment, the hERG peak tail currents were blocked by 48% at +20 mV. Normalized activation curves are shown in Fig. 2D. Propranolol caused no significant change in the half-maximal activation voltage ($V_{1/2}$) that were −13±0.1 mV and −12±0.2 mV (n=5) in control conditions and during application of 10 μM propranolol, respectively.

4. Discussion

We have demonstrated that propranolol, a β-adrenergic blocking agent, inhibits the hERG potassium channels. Potassium efflux through hERG is a major determinant of repolarization and action potential duration in ventricular cardiomyocytes. Blockade of hERG channels lengthens action potential duration and leads to QTc interval prolongation of the surface electrocardiogram. It is thought that QTc prolongation increases the likelihood of developing TdP, a life-threatening arrhythmia. We found that propranolol inhibited hERG in a concentration dependent manner with an IC_{50} of 9.9±1.3 μM indicating that propranolol has potential to induce QTc prolongation. In fact, QTc prolongation has been reported with intravenous propranolol under experimental conditions in humans (Milne et al., 1980). Propranolol has been in clinical use for over three decades for the treatment of a variety of diseases. The usual oral daily dosage range of propranolol is 80 to 320 mg, which achieves its therapeutic plasma concentrations of 77–193 nM and its maximum total plasma concentrations of 189±31 nM (Hardman and Limbird, 2001). The IC_{50} value of hERG inhibition by propranolol obtained in this study is about 300–

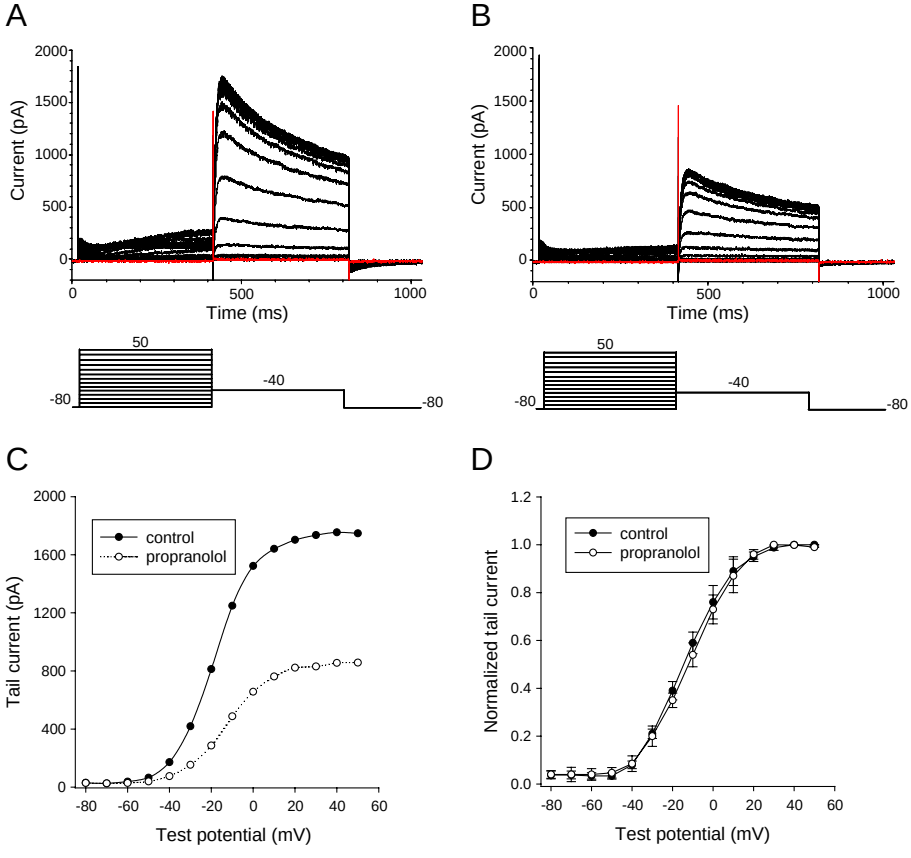

Fig. 2. Application of propranolol has no effect on hERG activation kinetics. Original current traces elicited from a cell before (A) and after exposure to 10 μM propranolol (B). Cell was held at a potential of −80 mV and depolarized to voltages in 10 mV increments at 25±0.5 °C. *Inset*: voltage-clamp protocol. *C*: *I–V* plot of the peak tail currents measured from the same cell before and after propranolol application. D, activation curve, *i.e.* the normalized peak tail current amplitude as a function of the test pulse potential, before and after propranolol application. The half-maximal activation voltage ($V_{1/2}$) is −13±0.1 mV and −12±0.2 mV, respectively.

530 fold higher than its human maximum free plasma concentration in humans at therapeutic doses, when a plasma protein binding of 87% is taken into account (Hardman and Limbird, 2001). Therefore, propranolol is unlikely to induce QTc prolongation in its therapeutic dose range (Redfern et al., 2003). However, in a case report, QTc prolongation occurred when propranolol was overdosed at 1600 mg by a 15-year old girl who tried to commit suicide (Farhangi and Sansone, 2003). The plasma levels were not measured in this case study; therefore, we calculated the levels using the pharmacokinetic data obtained from Ducret and Zech (1978), Borgstrom et al. (1981), and Kopitar et al. (1986). Based on these calculations, the maximum free and total plasma concentration of this overdosed propranolol were estimated to be 1.90 and 14.6 μM, respectively, which was close to the IC_{50} value of hERG inhibition by propranolol.

It is important to point out that prolongation of QTc in humans could also be due to the modulation of other cardiac ion channels, e.g. I_{Ks} or I_{Na} (Chiang and Roden, 2000). Since there is no detailed evidence in literature regarding the effects of propranolol on I_{Ks} or I_{Na}, we cannot rule out their possible involvement in the QTc prolongation in the case report (Farhangi and Sansone, 2003). Nonetheless, the ion channel most frequently implicated in cases of drug-induced QTc prolongation is hERG (Redfern et al., 2003).

In conclusion, the present results demonstrate that propranolol blocks hERG potassium channels. hERG inhibition could be a molecular mechanism for the previously reported QTc prolongation that was observed when propranolol is overdosed.

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